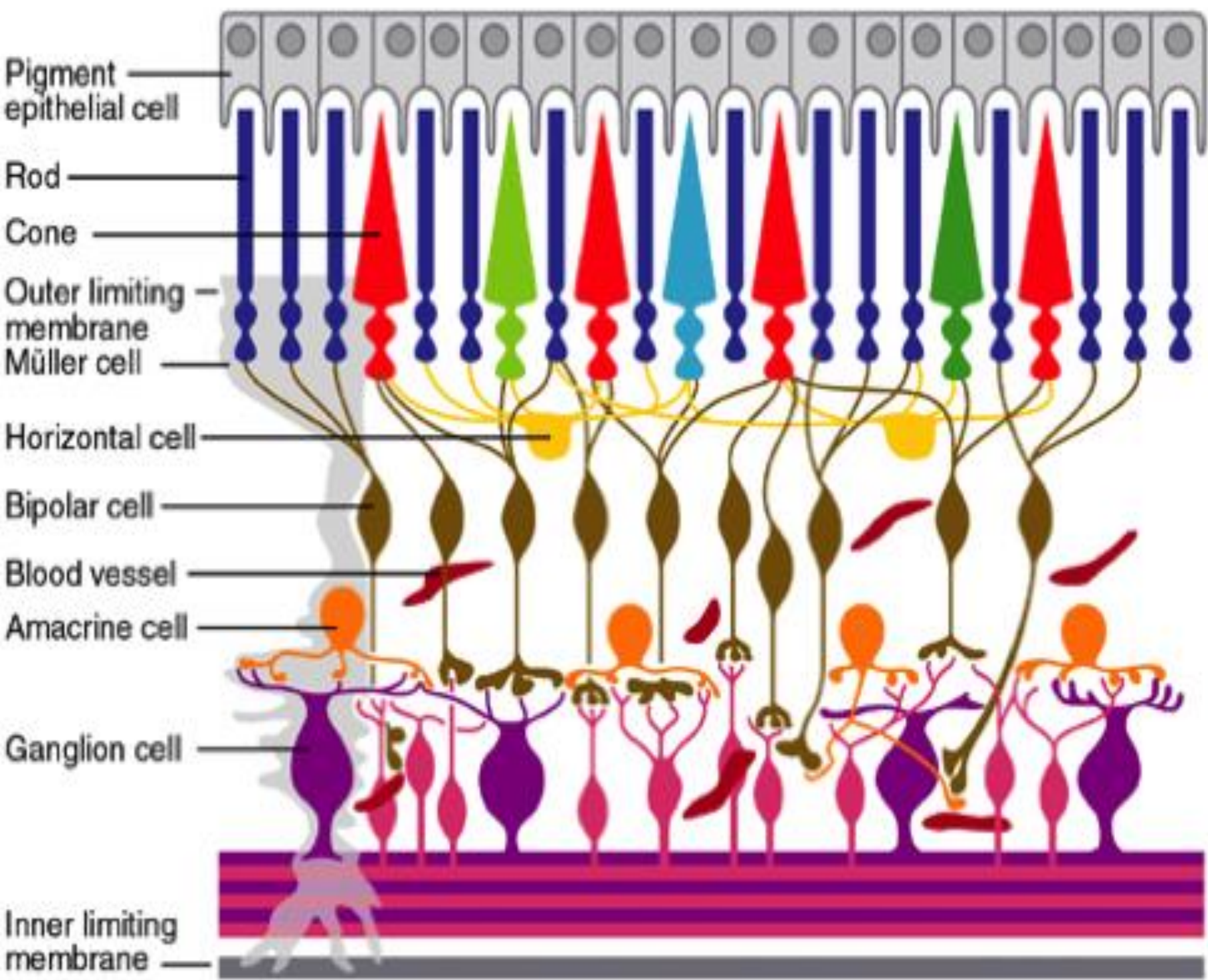


Deciphering the genetics of retinal ganglion cell development using CRISPR/Cas9 genome editing in zebrafish

Sanjeeva Weerasinghe*, Rebecca Chowdhury, and Jeffrey Trimarchi

Introduction Retinal ganglion cells are specialized nerve cells in the eye, which are involved in the processing of visual information. These specialized nerve cells develop from progenitor cells following an intricate pattern of gene expression. In this project, my goal was to elucidate the roles of several genes in the development of retinal ganglion cells in zebrafish. Specifically we have chosen genes from our single cell expression data that were found in developing ganglion cells and that encode proteins which modify histones. To address these questions, we first set out to determine the retinal expression of different SET domain containing proteins using in-situ hybridization. I generated a large number of different probes, but to date we have only observed widespread expression of the mRNAs. Second, I utilized the CRISPR/Cas9 system to generate mutations in different SET domain proteins with the goal of assessing the retinal phenotype of the mutants. I designed and generated numerous gRNAs, which were injected into fish at the one-cell stage. However, our mutation rates were mostly low in F0 fish, not allowing for any phenotypic analysis. These fish will need to be raised and crossed for generations to produce homozygote fish before the role of these proteins in retinal ganglion cell development can be ascertained.

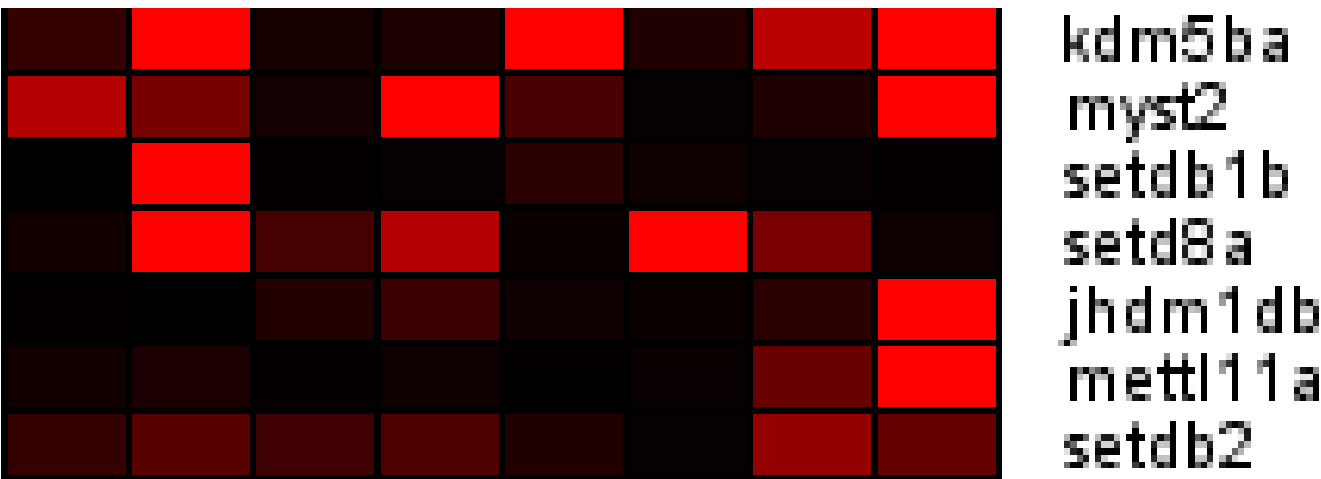


Cazals, F. & Kornprobst, P. 2004

This schematic shows the different cells layers of the retina. We are attempting to investigate which genes enable the proliferation of retinal ganglion cells found on the bottom of the retina.

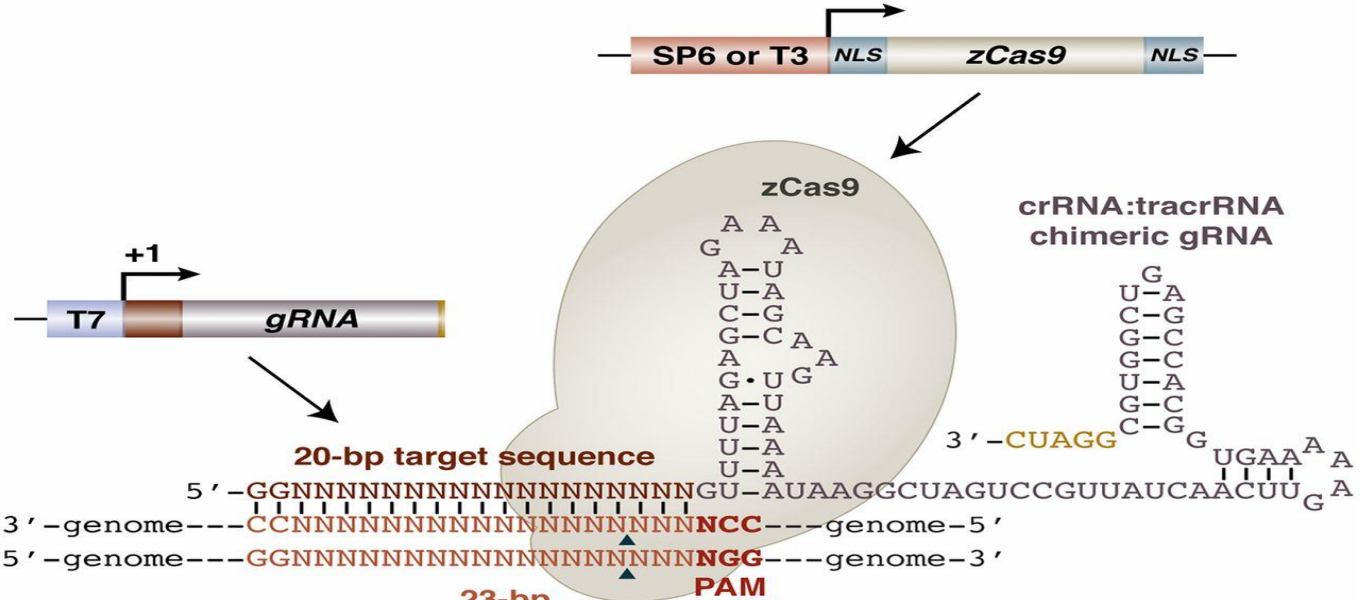
Single cell transcriptomics reveals cells genes potentially involved in retinal ganglion cell

50 hpf(hours post fertilization)

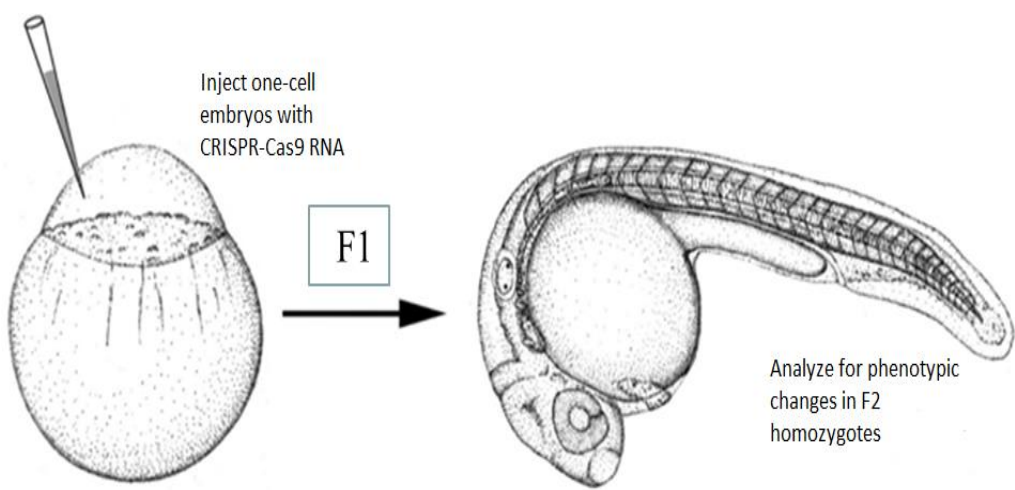


The heat map indicates that kdm5ba,mysT2,setdB1b,jhdm1db, and mettl11a are expressed at high quantites in the embryo. Red areas indicate areas of increased gene expression and black ones indicate areas of decreased expression.

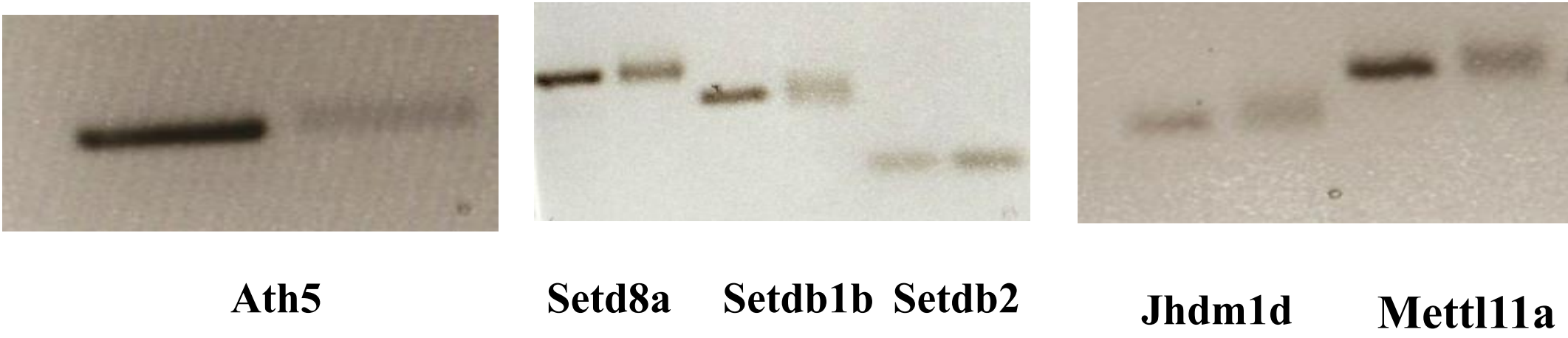
CRISPR/Cas9 cleaves genomic DNA at a specific target sequence



- Schematic above demonstrates how a CRISPR functions
- The figure below shows how the CRISPR is injected into a zebrafish embryo
- The bottom figure shows where the SetdB2 CRISPR mutates the SetdB2 gene.

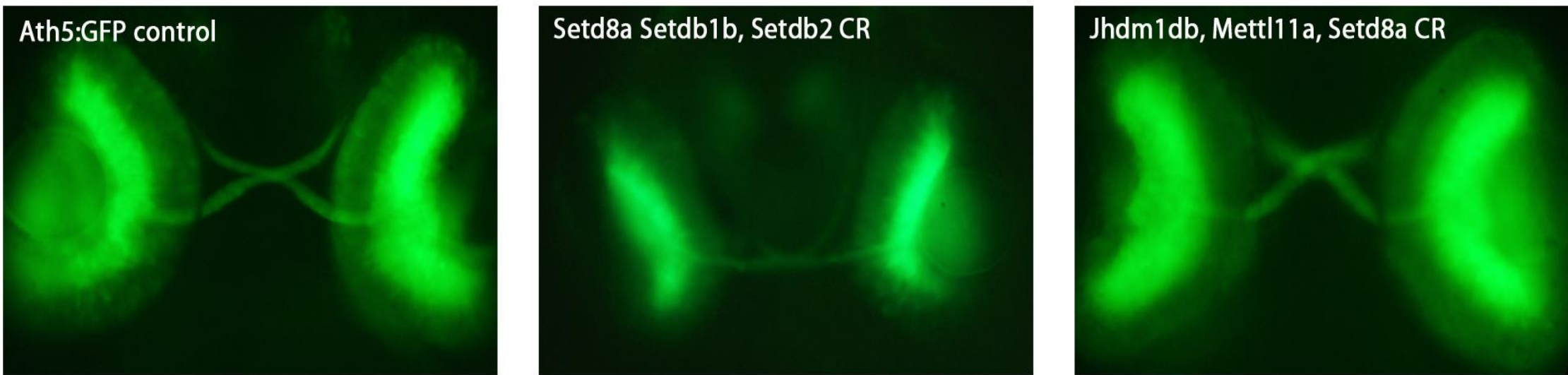


Generation of CRISPR mutants



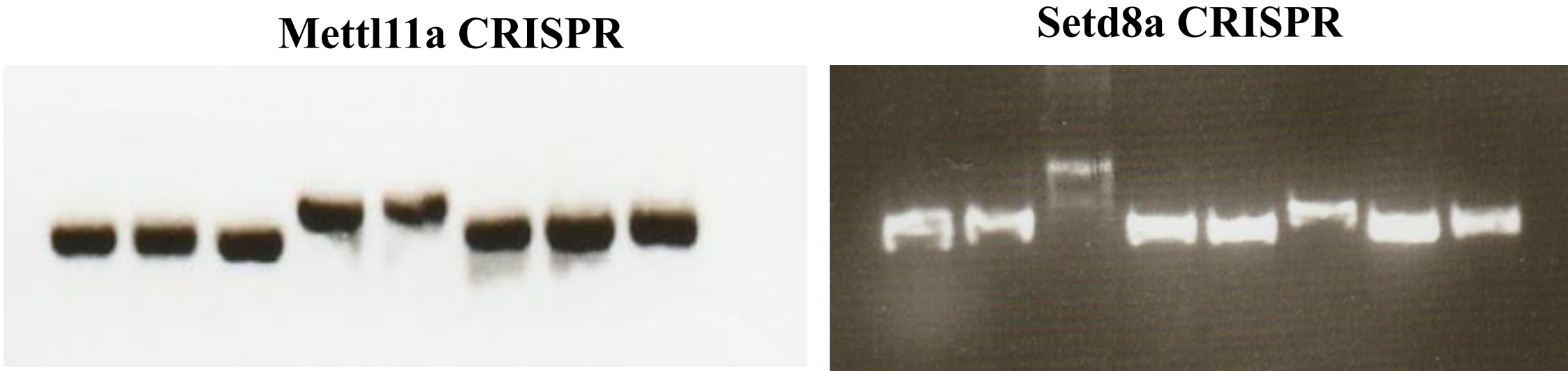
In each figure, the solid left band shows a wild type gene whereas the right band indicates the gene extracted from a fish injected with the corresponding CRISPR.If a smear is exhibited on the right band, then the CRISPR yielded a mutation in the corresponding gene.

Changes in optic nerve structure following CRISPR /Cas9 injection



- The control phenotype exhibits two normal optic nerves crossing each other
- The Setd8a, SetdB1b, SetdB2 KO fish exhibit two optic nerves crossing each other
- Likewise, the Jhdm1db, Mettl11a, and Setd8a KO fish exhibit two optic nerves crossing each other.

After we injected into F0s and did not see much of a phenotype (or any probably).We wanted to know why so we looked at the how many alleles were mutated (estimated).



In the case of Mettl11a, the CRISPR only mutated 2/8 alleles. The Setd8a CRISPR also yielded the same 2/8 mutation rate

Conclusions

- I successfully generated a number of CRISPR gRNAs which were then injected into zebrafish eggs of Ath5:GFP fish.
- We did not observe phenotypes in F0 injected fish.
- This was because the number of mutated alleles was usually 50% or below.
- Injected fish need to be raised and crossed for generations to produce homozygote recessive fish.

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